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SOLID-LIQUID EQUILIBRIUM DATA OF AMOXICILLIN AND HYDROXYPHENYLGLYCINE IN AQUEOUS MEDIA

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Abstract -The enzymatic synthesis of amoxicillin is catalyzed by Penicillin G Acylase (PGA). As byproducts, hydroxyphenylglycine and alcohol are also formed from hydrolytic reactions and antibiotic synthesis, respectively. The design of this process should be directed to promote the synthesis reaction. At the same time, it is necessary to reduce the hydrolytic reaction of amoxicillin through its crystallization or separation from the reaction medium. This work presents measurements of solid-liquid equilibrium data for amoxicillin and hydroxyphenylglycine in water at different temperatures (283.15 – 298.15 K), pH (5.5 – 7.5) and ethanol composition (0 – 70 wt.%). This information is relevant to determine the conditions that offer the lowest solubility for the antibiotic, favoring its separation and purification. All solubility data were obtained using an analytical method with indirect determination by UV spectroscopy. Ideal thermodynamic modeling was applied to describe the experimental solubility data sets.
Keywords: Amoxicillin; Hydroxyphenylglycine; Solubility; Crystallization; Enzymatic synthesis.

INTRODUCTION

Penicillin represents one of the most important groups of β-lactam antibiotics, being the most prescribed in medicine. Its discovery is still a landmark in the treatment of bacterial infections, especially amoxicillin (AMOX), a semi-synthetic penicillin and the drug most consumed in Brazil according to the Ministry of Development, Industry and Foreign Trade.

The conventional route adopted for the production of β-lactam antibiotics is by chemical synthesis, a complex process that requires several reaction steps, low temperature, anhydrous conditions and the use of highly toxic solvents, generating considerable amounts of non-biodegradable wastes (Ospina *et al.,* 1996).

The enzymatic processes offer important advantages over these conventional processes and are becoming of greater relevance, since the reactions can be performed in a single step with high yield and specificity, under mild conditions, minimizing or eliminating waste and avoiding the use of toxic solvents (Ospina *et al.,* 1996; Diender *et al.*, 1998, Hernández-Jústiz *et al.*, 1999; Wegman *et al.,* 2001).

Among the enzymatic approaches, the kinetically controlled route shows feasibility for the synthesis of antibiotics derived from α-amino acids (Youshko *et al*., 2000), which uses an activated side chain donor (e.g., an ester or amide) and the penicillin nucleus,

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6-aminopenicillanic acid (6-APA). In the enzymatic synthesis of amoxicillin from hydroxyphenylglycine methyl ester (HPGM) and 6-APA, as indicated in Figure 1, the Penicillin G Acylase (PGA) catalyzes both the synthesis of the antibiotic by coupling (Reaction 1) and the undesired hydrolysis of the substrate (Reaction 2) and its product (Reaction 3), generating hydroxyphenylglycine (HPG) and alcohol. Therefore, the yield of the process depends on the kinetic rates of these three reactions, where the antibiotic acts as the intermediate component within a set of series-parallel reactions (Santana *et al*., 2010). Thus, the concentration curve of antibiotic reaches a maximum value, beyond which the hydrolytic reactions prevail.

Since the enzymatic reaction occurs in the liquid phase, a way to shift the equilibrium towards the synthesis of amoxicillin is by decreasing its concentration in the aqueous medium by reducing its solubility. This can be performed by changing the conditions of pH, temperature and ethanol composition. Thus, the antibiotic in excess in relation to its solubility will be transferred to the solid phase by crystallization and will not be available for hydrolysis, increasing significantly the productivity and selectivity of the process. Solid-liquid equilibrium data, or solubility measurements, are therefore an important tool for the description of the behavior of the compounds involved in the enzymatic synthesis of amoxicillin, since solid and liquid equilibrium is the basis for separation processes, e.g., crystallization.

As shown in Figure 1, both AMOX and HPG have amino acid characteristics, i.e., have amino and carboxyl groups in their structures. Thus, they have two ionization sites with different affinities for protons, which can undergo protonation and deprotonation. Due to this fact, molecules can be present in three distinct forms of ionization, i.e., anionic, cationic or zwitterionic, in different concentrations according to the pH of the solution, known as speciation. This is because the lower the pH value, the greater the concentration of H^+ ions in solution, thereby prevailing positively charged species. In contrast, the higher the pH value, the lower the amount of H^+ ions in solution, with prevalence of negatively charged species. In an intermediate pH condition there will be an electrically neutral form, but with both anionic and cationic groups present in the molecule. These molecules, where there is an equivalence between the positive and negative charges, are called *zwitterions*, and the pH where the molecule has no net charge is called the isoelectric point. In order to assist in the separation step of the antibiotic it is relevant to know these characteristics, since the solubility of these molecules is due largely to the distribution of their electrical charges.

Literature measurements concerning the solid-liquid equilibrium of β-lactam antibiotics are scarce (Santana *et al.*, 2010). Diender *et al.* (1998) determined the solubility of HPG, 6-APA and AMOX in water as function of pH at 298.15 K according to the analytical method proposed by Gude *et al.* (1996). Using this method, Vieira (2003) determined the solubility of phenylglycine methyl ester (EMPG), ampicillin (AMPI), 6-APA and phenylglycine (PG) in water at 298.15 K as a function of pH. The results showed that increasing the pH leads to an increase in the solubility of 6-APA and ampicillin. In contrast, EMPG showed a decrease in solubility with increasing pH, while PG retained its solubility limit practically constant.

Rudolph *et al*. (1999) presented experimental data for the solubility of ampicillin, amoxicillin and their precursors in water in a range of temperature at the isoelectric point and varying the pH at 298.15 K. The experimental data were successfully described using a simplified model proposed by Khoshkbarchi and Vera (1996).

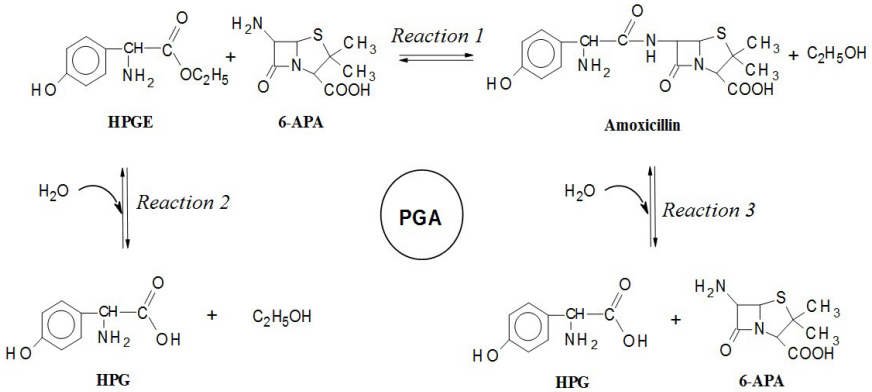

Figure 1: Reactions involved in the kinetically-controlled synthesis of amoxicillin

Youshko *et al*. (2000) measured the solubility of individual components of the reaction of enzymatic synthesis of ampicillin in the pH range between 4 and 8 at 298.15 K. From these measurements, it was concluded that the precipitation of PG is almost inevitable in the course of the reaction when using high initial concentrations of substrates at any value of the pH. The optimal conditions according to these data were found to be in the range of pH between 6 and 7, the precipitation of ampicillin being effective only at pH below 7.5.

Santana *et al.* (2010) determined the solubility for a series of the components involved in the enzymatic synthesis of ampicillin at different values of temperature and pH, using the saturation method proposed by Gude *et al*. (1996) and modified by Vieira (2003). In order to describe the measured solubilities, the model of Khoshkbarchi and Vera (1996) was applied.

Due to the complexity of biomolecules, i.e., their multiple functional groups and the crucial role of water, new thermodynamic models for describing the phase behavior of systems containing biomolecules are required for the process description. In recent years, several research groups have investigated the phase behavior of systems containing amino acids and proteins. However, there are few thermodynamic models available in the literature describing the solubility of antibiotics.

Franco and Pessôa Filho (2011) showed thermodynamic relations between the protein solubility and the pH of the solution. The chemical potential of the protein in the liquid phase can be described by Henry's law and the solid-liquid equilibrium is established only between neutral molecules. The mathematical development resulted in an analytical expression for the solubility curve as a function of the ionization constants, pH and the solubility of the compound at the isoelectric point, being applied with success in the description of the solubility of porcine insulin as a function of pH at three different temperatures and bovine β-lactoglobulin at four different ionic strengths.

In order to elucidate the behavior of this property in the face of the various possible conditions of the process, this work has focused on collecting data for AMOX and HPG solubility in water covering a pH range from 5.5 to 7.5 in the temperature range between 283.15 and 298.15 K. As there is formation of alcohol as a byproduct of the synthesis, the effect of different ethanol concentrations on the solubility of HPG was also examined at 298.15 K. Finally, a thermodynamic model was used to describe mathematically the solubility curves obtained experimentally.

MATERIALS AND METHODS

Materials

HPG 98% and AMOX 94% (purity checked by Total Organic Carbon analysis - TOC) were obtained from Sigma-Aldrich and used without further purification. NaOH of analytical grade was obtained from CIRQ and analytical grade ethanol was from Merck. In all experiments deionized ultra-pure water was used obtained by passing distilled water through an ion exchange column. The measured values of purity were used in the analytical procedure to correct the values of composition given by the calibration curve.

The pH measurements were performed using a Metrohm model 827 pH-lab pHmeter equipped with a glass electrode combined with a temperature sensor. The absorbance readings were obtained on a Varian Cary 50 model spectrophotometer, using a quartz cuvette. The temperature of the equilibrium measurements was controlled by a Tecnal, model TE-184 thermostatic bath.

Methods

Solubility of the Components

The solubilities of AMOX and HPG in pure water were determined experimentally using the analytical method of saturation. The saturated solutions were prepared with an excess of solute, ensuring the presence of the solid phase. The desired value of the pH was adjusted by addition of aqueous 0.1 M NaOH or 0.1 M HCl solution. This mixture was prepared in a jacketed equilibrium cell (40 mL) which is provided with temperature control by circulating water from the thermostatic bath. The cell also has a magnetic spin bar to provide stirring of the mixture. The solubility procedure was based on the reproducibility of two sample concentration. For each sample, or assay, the mixture was subjected to three hours of stirring followed by two hours of sedimentation of undissolved solids. Then a small aliquot of the supernatant was withdrawn and diluted for analysis of the solute concentration in a spectrophotometer in the region of the ultraviolet. For AMOX, the dilution factor used was 1:50 and for HPG, 1:400. Both components showed peak

absorption at λ=270 nm, which is the wavelength used in the determination of absorbance and the concentration from a calibration curve for each component. To confirm that the equilibrium was reached, the remaining solution in the cell was again subjected to agitation for three hours and then left to stand for two hours and finally the analysis repeated for the acquisition of the duplicate. The solubility was determined based on the reproducibility of these two successive samples.

For the experiments involving the mixture of water and ethanol, saturated solutions were initially prepared with an excess of undissolved solute in mixtures with different proportions by mass of ethanol, and then subjected to the same procedure described previously. The dilution factor for samples containing 10, 30, 50 and 70% ethanol were 1:400, 1:400, 1:200 and 1:50, respectively.

Dissociation Constant

The pK values were determined using potentiometric titration with the aid of the equilibrium cell. Stock solutions of 2 mM AMOX and 5 mM HPG were used for these experiments. Initially, 10mL of stock solution of the component of interest was added to the equilibrium cell under constant magnetic stirring and at a fixed constant temperature. The temperature was adjusted to the value of interest with the aid of a thermometer sensor placed in the solution, and circulating water from the thermostatic bath. After stabilization of the solution temperature, the solution was acidified by adding aqueous 0.1 M HCl to a pH around 2.5 and then subjected to dropwise titration using 0.1 M NaOH as titrant with the pH being measured after each drop addition with a pHmeter. The pK values were determined from changes in the shape of the titration curve by evaluation of the first and second derivative curves.

Mathematical Modeling

As the functional side chains of β-lactam antibiotics are comparable to the functional side chains of amino acids (Rudolph *et al*., 2001), this work assumed the same approach used for systems containing proteins to model the phase behavior of systems containing antibiotics.

Assuming that the phase equilibrium occurs only between the neutral molecules of the solute, the molality of these molecules in the liquid phase must remain unchanged with variation of the pH of the solution, being a fraction of the solubility. Therefore, Equation (1) may be derived from the equality of the molalities of the neutral species.

$$S(pH) = \frac{\phi_0(pH)S(pI)}{\phi_0(pI)} \tag{1}$$

where ϕ_0 is the fraction of electrically neutral solute molecules in the liquid phase and S is the solubility in the liquid phase. Franco and Pessôa Filho (2011) obtained Equation (2) for calculating the electrically neutral molecular fraction, which was used in this study to predict the solubility of the antibiotic, considering ideal behavior in the liquid phase.

$$\log\frac{S(pH)}{S(pI)} = (pI - pH) + \log\left(\frac{1+10^{pH-pK_1}}{1+10^{pI-pK_1}}\right) + \log\left(\frac{1+10^{pH-pK2}}{1+10^{pI-pK_2}}\right) \tag{2}$$

To examine the validity of the ideal model for the description of the experimental data, the relative and average deviations were determined by Equations (3) and (4).

$$\Delta S(\%) = \left(\frac{S_{exp} - S_{calc}}{S_{exp}}\right).100 \tag{3}$$

$$\overline{\Delta S}(\%) = \frac{\sum|\Delta S(\%)|}{N} \tag{4}$$

where ΔS(%) is the relative deviation, $\overline{\Delta S}(\%)$ is the absolute average deviation, S_{exp} is the solubility determined experimentally, S_{calc} is the solubility determined by the thermodynamic model, and N is the amount of data.

RESULTS AND DISCUSSION

The solubility results obtained for AMOX and HPG in pure water and their standard deviations calculated at different temperatures and pH are presented quantitatively in Table 1 and plotted in Figures 2 and 3. The low standard deviation values indicate the accuracy of the measurements, which are in agreement with the analytical accuracy. In order to present the order of magnitude of the solubility uncertainties, all plots were prepared with error bars.

Table 1: Solubility data of amoxicillin and hydroxyphenylglycine in water at different temperatures and pHs

Amoxicillin			Hydroxyphenylglycine		
pH	Solubility (mM)	S.D. (mM)	pH	Solubility (mM)	S.D. (mM)
T = 298.15K					
5.56	6.42	0.08	5.53	115.04	1.10
5.89	6.57	0.02	6.09	113.37	0.37
6.58	7.73	0.09	6.60	115.03	0.92
7.23	13.10	0.01	7.08	115.11	1.32
7.58	23.16	0.21	7.45	119.22	0.83
T = 293.15K					
5.61	5.89	0.05	5.47	110.32	1.74
6.01	6.57	0.02	6.16	110.00	1.61
6.65	6.81	0.07	6.64	110.81	1.56
7.10	10.60	0.07	7.02	110.97	2.04
7.52	22.74	0.22	7.56	114.08	0.55
T = 288.15K					
5.51	5.54	0.30	5.59	104.79	1.26
6.09	5.36	0.25	6.06	103.70	3.09
6.68	6.55	0.17	6.67	104.43	1.94
7.07	9.71	0.12	7.04	104.96	2.13
7.69	22.56	0.01	7.53	110.46	2.73
T = 283.15K					
5.69	4.67	0.01	5.58	97.42	1.71
6.17	5.02	0.17	6.11	98.38	0.12
6.88	6.51	0.02	6.68	99.19	0.79
			7.13	100.11	0.59
			7.56	101.52	1.36

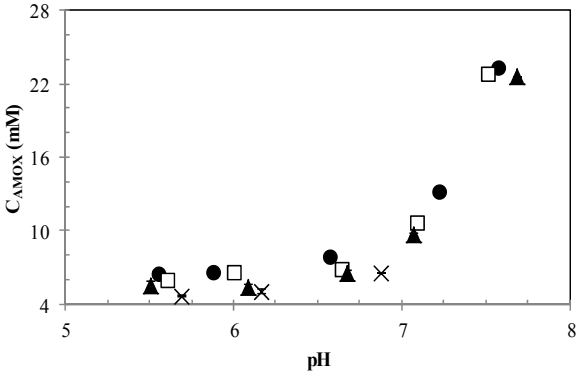

Figure 2: Solubility of amoxicillin in water as a function of pH at different temperatures (K): ● 298.15; □ 293.15; ▲ 288.15; × 283.15.

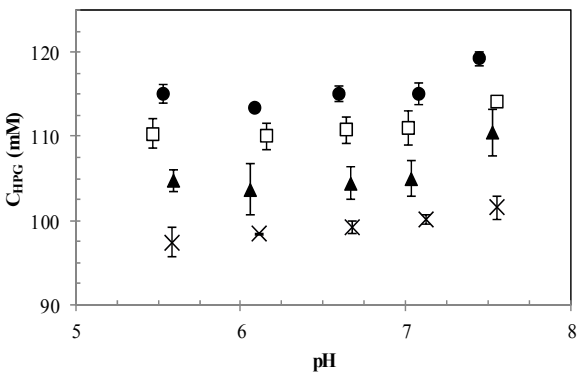

Figure 3: Solubility of hydroxyphenylglycine in water as a function of pH at different temperatures (K): ● 298.15; □ 293.15; ▲ 288.15; × 283.15.

The temperature showed no significant influence on the solubility of this compound, with an average increase of about 0.44 mM for each increment of 5 K in the same pH range.

In contrast, the solubility of HPG showed the opposite behavior, remaining practically constant with the variation of pH, as found in the literature (Diender *et al.,*1998; Rudolph *et al.,* 2001). However, the solubility of HPG showed much more temperature dependence, i.e., a decrease of 6 mM for each 5 K decrease, approximately. According to Petsev *et al.* (2001), the small influence of temperature on solubility of the compounds indicates that the crystallization enthalpy of these approaches zero, whereas the entropy is independent of temperature, or weakly dependent.

Besides this difference in behavior, it is important to note the differences in magnitude of the solubility in water between these two compounds, which are related to the hydrophobicity of the solute. According to Rudolph *et al*. (1999) amoxicillin is almost two times more hydrophobic than hydroxyphenylglycine. Furthermore, HPG has a lower molecular mass and smaller chemical structure than AMOX, favoring its solubility in water.

Solubility data for AMOX and HPG in water were found in the literature only at 298.15 K. The results obtained in this work showed satisfactory agreement with the literature data, as can be observed in Figure 4.

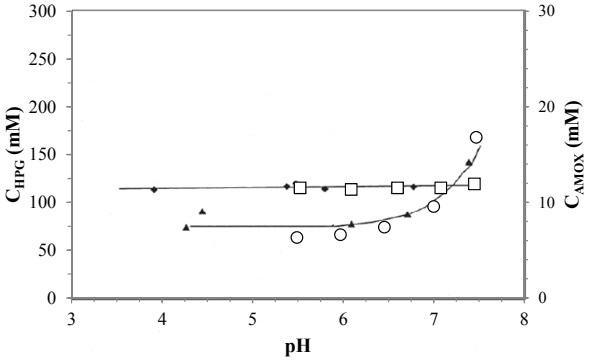

Figure 4: Comparison of the solubility in water at 298.15K: (▲ and ○) AMOX; (◆ and □) HPG. Full: Diender *et al.* (1998), and empty: this work

Table 2 and Figure 5 present the solubility data obtained experimentally for HPG at 298.15 K as a function of ethanol content, with their standard deviations, respectively. The solubility of HPG decreases dramatically with the ethanol concentration, with an average of 115 mM in pure water and reducing to 15 mM in the medium containing 70 wt.% ethanol. This solubility (15 mM) is of the same order of magnitude observed for AMOX in water at pH values above 7. The reduction in the solubility with organic solvent concentration may be explained by the fact that the addition of ethanol provokes a decrease in the dielectric constant and water activity of the medium. According to Douhéret and Pall (1988) and Franks (1973), the dielectric constants of water and ethanol are 78.35 and 24.60, respectively. This means that ethanol has a lower ability to dissolve polar solutes than water. The addition of ethanol promotes aggregation by electrostatic interactions between the solute molecules.

Concerning the pH variation, the same influence on the solubility was observed in the solvent mixture as in pure water, remaining constant in the pH range studied.

Table 2: Solubility data of hydoxyphenylglycine in ethanol + water mixtures at 298.15K as a function of pH

pH	Solubility (mM)	S.D. (mM)
0 wt.% of ethanol		
5.53	115.04	1.10
6.09	113.37	0.37
6.60	115.03	0.92
7.08	115.11	1.32
7.45	119.22	0.83
10 wt.% of ethanol		
5.52	91.91	0.15
5.97	93.02	0.87
6.45	92.95	0.97
6.97	94.72	0.46
7.51	95.55	0.12
30 wt.% of ethanol		
5.57	52.67	0.41
6.01	53.27	0.71
6.57	54.74	1.81
6.99	55.53	0.01
7.46	58.38	0.11
50 wt.% of ethanol		
5.54	33.28	0.58
6.10	22.92	0.14
6.57	33.60	0.01
6.96	34.75	0.26
7.35	35.28	0.39
70 wt.% of ethanol		
5.60	16.13	0.23
5.96	14.50	0.10
6.48	13.97	0.37
7.02	15.64	0.10
7.47	16.03	0.04

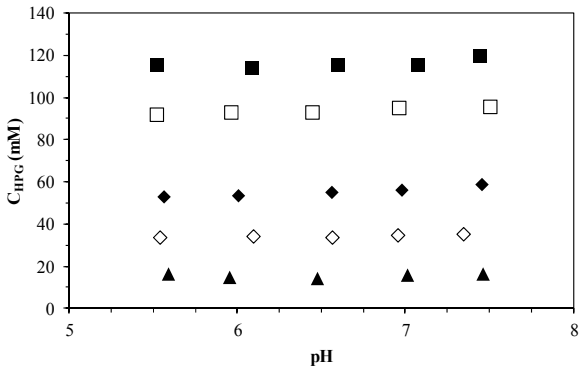

Figure 5: Solubility of hydroxyphenylglycine in ethanol + water mixtures at 298.15K as a function of pH at different compositions of ethanol (wt.%): ■ 0; □ 10; ◆ 30; ◇ 50 and▲ 70.

Table 3 gives the values of the ionization constants and isoelectric point of AMOX and their standard deviations. Table 4 presents ionization constants from the literature for comparison. As the carboxylic group is more acidic than the amino

group, their dissociation is represented by the first equilibrium constant (pK_1), the second equilibrium constant (pK_2) resulting from dissociation of the amino group. It may be seen that there is a slight antagonic tendency between the dissociation constants (pK_1 and pK_2) with temperature. Thus, as this parameter increases, the carboxyl group decreases its acid character and the amino group becomes more acid, decreasing the range of predominance of the zwitterionic molecules.

Table 3: Experimental isoelectric points and dissociation constants of amoxicillin at different temperatures

Temperature (K)	pK_1	S.D.	pI	S.D.	pK_2	S.D.
298.15	2.74	0.257	4.86	0.156	6.98	0.055
293.15	2.74	0.087	4.92	0.069	7.10	0.060
288.15	2.72	0.144	4.97	0.128	7.22	0.111
283.15	2.66	0.095	4.97	0.030	7.28	0.020

Table 4: Comparison between dissociation constants obtained in this work in water and data from the literature at 298.15 K

Reference	HPG		AMOX	
	pK_1	pK_2	pK_1	pK_2
This work	-	-	2.74	6.98
Diender *et al.,* 1998	2.20	9.20	2.90	7.40
Rudolph *et al.,* 1999	1.96	9.02	2.63	7.16
Ulinj *et al.*, 2001	2.5	9.2	-	-

Given the values of pK_1 and pK_2, the speciation of the different ionic forms of the compounds in solution as a function of pH can be performed, as described by Sandler (2006) for the ionization of phthalic acid. At pH values below pK_1, it is known that there are predominantly positively charged molecules and at pH values above pK_2, there are predominantly negatively charged molecules. Due to the presence of an excess of electric charges in these regions there are considerable increases in solubility. In the range between the two dissociation constants, there is generally a buffer region, wherein the pH influences only slightly the solubility of the compound due to the predominance of zwitterionic species.

In the potentiometric curve of HPG in pure water there was only one point of inflection, representing its isoelectric point (pI), instead of two inflection points, as expected. Therefore, potenciometric titration was not able to determine the values of both pK_1 and pK_2 for HPG in water. This may be explained by the low acidity of the amine group present in HPG (Pereira *et al.*, 2011). Capillary zone electrophoresis (CZE) and ionic mobility curves may be applied to determine pK_1 and pK_2 values of weak acids (Slampová *et al.*, 2008). pI values for HPG in pure water at different temperatures are reported in Table 5.

Table 5: Experimental isoelectric points of hydroxyphenylglycine at different temperatures

Temperature (K)	298.15	293.15	288.15	283.15
pI	5.38	5.48	5.49	5.51
S.D.	0.110	0.021	0.031	0.032

On the other hand, using aqueous ethanol media it was possible to determine pK values. The second equivalence point (or inflection point) was observed in the potentiometric curves of these systems at 298.15 K with the increase of the composition of ethanol. For instance, at 30 wt.% of ethanol pK_1 and pK_2 were 2.67 and 8.87, respectively, which are close to the literature values. Thus, it was possible to determine both pK_1 and pK_2 values for the systems containing HPG and 30, 50 and 70 wt.% of ethanol. The results are listed in Table 6.

Table 6: Experimental isoelectric points and dissociation constants of hydroxyphenylglicine at different ethanol concentrations

[Ethanol] (wt. %)	pK_1	S.D.	pI	S.D.	pK_2	S.D.
10	-	-	5.43	0.09	-	-
30	2.67	0.06	5.77	0.01	8.87	0.03
50	2.95	0.08	6.04	0.03	9.13	0.01
70	3.16	0.05	6.24	0.02	9.33	0.02

The carboxylic group of HPG is very acidic, corresponding to a low pK_1value. The amino group is very basic, resulting in a relatively high value of pK_2. Thus, HPG has a wide pH range in which there is a predominance of electrically neutral (zwitterionic) molecules. This results in a buffer solubility behavior. The pH values studied in this work ranged from the pI to a value below pK_2, where there is no predominance of positive or negative charged species. Therefore, it was not possible to observe a significant effect of pH on the solubility in the range studied.

The amine group of amoxicillin is more acidic than HPG, providing a pK_2 value even lower. The pH range studied in this work for AMOX ranges from values close to the pI up to values higher than pK_2, where there is practically only the negative species present. Thus, the amoxicillin data set showed a greater influence of pH on solubility compared to the HPG measurements.

Dissociation constant values of amoxicillin and hydroxyphenylglycine were determined at the different temperatures and compositions of ethanol studied. Then, it was possible to calculate the solubility of these compounds from the ideal model of solubility by Equation (2). Figure 6 presents a comparison of the AMOX solubility values with the ideal model as a function of pH at different temperatures. Figure 7 presents ideal thermodynamic modeling of the HPG solubility curve in mixtures of water and ethanol in different compositions and pH values.

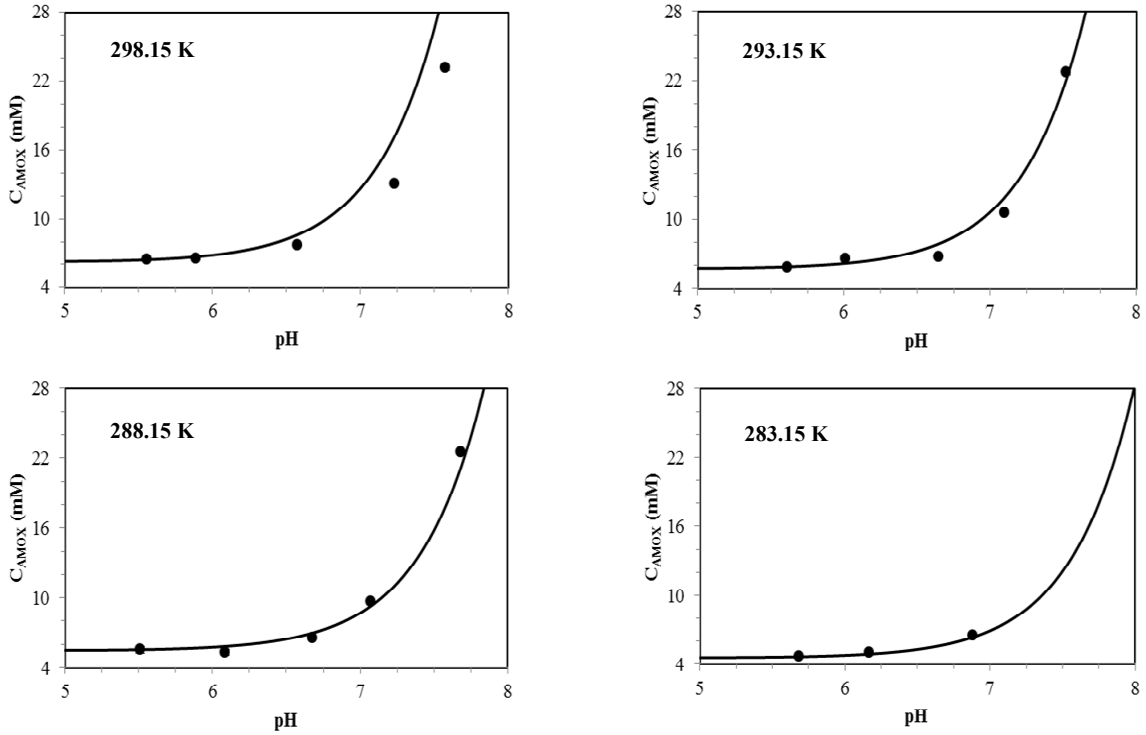

Figure 6: Solubility of amoxicillin in water as a function of pH at different temperatures: ● Experimental data; ▬ Ideal model.

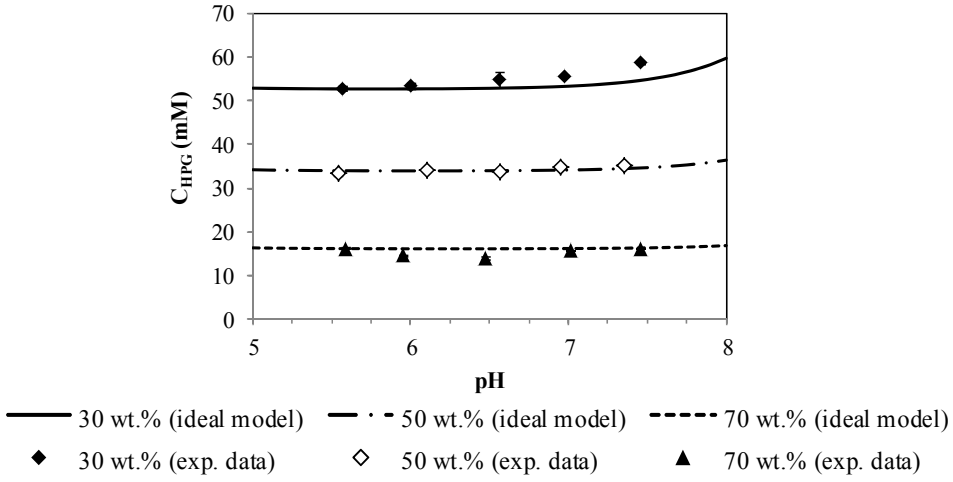

Figure 7: Solubility of hydroxyphenylglycine at 298.15 K in different ethanol compositions.

Agreement of the correlation with experiment is obtained, ensuring the applicability of the ideal thermodynamic model used in the treatment of the solid-liquid equilibrium of this system. However, according to Franco (2012), the validity of this model is restricted to the region close to the isoelectric point, since at pH values remote from this point, the fraction of neutral molecules tends to zero and Equation (1) would predict an infinite solubility, being physically unacceptable. Therefore, it is important to apply an activity coefficient model to describe non-idealities of the solution. However, the ideal assumption provides a reasonably accurate description of the solubility curve of β-lactam antibiotics.

Tables 7 and 8 present the deviations from the ideal model, which are significant but acceptable for process purposes. These deviations demonstrate the requirement of non-ideality correction to improve the data representation that may reach values of ca. 1.5 for the activity coefficient.

Table 7: Average deviation between experimental and calculated values of the solubilities of the studied compounds in water by the ideal model

Temperature	AMOX (%)	HPG (%)
298.15K	15.53	0.71
293.15K	1.18	-
288.15K	3.36	-
283.15K	14.26	-

Table 8: Average deviation between experimental and calculated values of the solubilities of HPG in mixture water and ethanol by the ideal model

Ethanol composition (wt.%)	HPG (%)
30	0.71
50	2.24
70	6.24

CONCLUSION

In this paper, new experimental solubility data for the system involved in the enzymatic synthesis of amoxicillin (AMOX and HPG) were presented. The solubility measurements for HPG show little influence of pH in the studied range, confirming the information given in the literature. The solubility of amoxicillin at 298.15 K was also in agreement with the literature (Diender *et al*., 1998). In addition, it was observed that ethanol has a significant effect on the precipitation of the compounds studied. In order to favor the purification of AMOX by crystallization, it is necessary to keep HPG in solution. It has been demonstrated by the measurements that the addition of ethanol reduces the solubility of HPG on the same order of magnitude of AMOX solubility in water, making this unfeasible for the separation. In this case, it is important to study the solubility of AMOX against various proportions of ethanol. The thermodynamic modeling applied describes the experimental solubilities satisfactorily for process purposes. For a more rigorous approach, it is necessary to taken into account the nonidealities by an activity coefficient model, i.e., issues for further work.

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